Infusion of reconstituted high-density lipoproteins (rHDL) is being studied in clinical trials as an adjunctive therapy for gram-negative sepsis. Since no data are available on its possible effects in systemic candidiasis, we investigated the effect of rHDL infusion into volunteers on the growth of Candida albicans. C. albicans growth was 10- to 100-fold higher in the plasma of volunteers infused with 80 or 100 mg/kg rHDL than in plasma collected before infusion; administration of 60 mg/kg rHDL had marginal effects. In vitro, the isolated lipoprotein subfractions had a growth-promoting effect on C. albicans. These data suggest potential adverse effects of rHDL if infused into patients with systemic candidiasis. Thus, rHDL infusion into patients with sepsis caused by an unknown microorganism may be contraindicated.

Despite the availability of new and potent antibiotics, mortality from gram-negative bacterial sepsis has remained constant during the last 30–40 years [1]. The lipopolysaccharide (LPS) component of the gram-negative bacterial cell wall mediates most of the inflammatory actions induced by these microorganisms, and conceptually, its neutralization could have beneficial effects on the outcome. Recent studies have applied reconstituted high-density lipoproteins (rHDL) for binding and neutralization of LPS. It has been shown that rHDL administered to animals protects against lethal endotoxemia [2] and gram-negative sepsis [3]. In addition, its infusion into humans down-modulates the inflammatory reaction induced by LPS [4]. Because of these effects, it has been suggested to use rHDL infusion as an adjunctive therapy in gram-negative sepsis. However, sepsis can be caused by a variety of microorganisms, including gram-negative bacteria, gram-positive bacteria, and fungi, and on clinical grounds it is often impossible to distinguish between these causes. Therefore, it is important to know whether rHDL infusion would affect the course of sepsis due to microorganisms other than gram-negative bacteria.

Acute disseminated candidiasis is a serious infection, of which the incidence has increased at least 10-fold during the last decade [5]. We have recently shown that hypercholesterolemia in low-density lipoprotein (LDL) receptor–deficient mice increases the susceptibility of these animals to systemic candidiasis [6]. In addition, earlier studies have demonstrated that Candida albicans grows better in lipid-containing emulsions used for parenteral feeding than it does in standard preparations [7–9]. These data suggest that a lipid-rich environment promotes C. albicans growth, and this could be a serious drawback for empirical rHDL treatment. For the present study, we asked whether rHDL infusion into human volunteers leads to increased candidal growth. In addition, we investigated whether freshly isolated human lipoproteins promote the growth of C. albicans in vitro and whether C. albicans grows better in plasma of hyperlipoproteinemic mice than in control plasma.

Materials and Methods

**Infusion of rHDL into human volunteers.** Three groups of four healthy volunteers each were infused with rHDL at various doses (60, 80, or 100 mg/kg) over a 4-hour period. Blood samples were collected just before the infusion (time 0) and 4, 12, and 24 hours after the start of the infusion. Plasma samples from subjects receiving the same dose were pooled at each time point and were provided by Dr. Jan Eva Doran and Dr. Alphonse Hubsch (ZLB Central Laboratory, Bern, Switzerland).

**Isolation of the lipoprotein subclasses.** LPS-free very-low-density lipoprotein (VLDL; final cholesterol concentration, 0.9 mmol/L), LDL (2.0 mmol/L cholesterol), and HDL (0.5 mmol/L cholesterol) subclasses, as well as lipoprotein-depleted plasma (LPDP) (<0.1 mmol/L cholesterol), were isolated by sequential ultracentrifugation from fresh EDTA-treated plasma of healthy volunteers who did not receive rHDL infusion. The methods for isolation of lipoprotein subclasses have been described earlier [10]. Lipoproteins were dialyzed for 24 hours against 0.05 mM phosphate buffer, pH 7.4, containing 5 mM EDTA/L, with one exchange of the buffer. Lipoprotein subfractions isolated from six volunteers were studied in triplicate.
Animals. Homozygous C57BL/6J mice lacking LDL receptors (LDLR−/−) and wild-type LDLR+/+ controls were obtained from Jackson Laboratories (Bar Harbor, ME) as mating pairs and bred in our local facility. LDL concentrations ± SD in the LDLR−/− mice were 4.92 ± 0.53 mmol/L, compared with 0.35 ± 0.11 mmol/L in control LDLR+/+ mice [11]. Blood was collected on EDTA from 6- to 8-week-old mice, and plasma was obtained for the use in C. albicans growth experiments. LPDP from LDLR−/− and control animals was prepared by ultracentrifugation, as described above. Plasma isolated from five LDLR−/− and five LDLR+/+ control mice was studied in three separate experiments.

C. albicans growth in vitro. C. albicans (American Type Culture Collection 10231; 10^4 cfu/mL) was grown in the pooled plasma of rHDL-infused volunteers, as well as in the various lipoprotein subfractions or lipoprotein-depleted plasma from untreated volunteers, after 1:1 dilution in Sabouraud broth with 2% glucose. Similarly, C. albicans (10^5 cfu/mL) was grown in a 1:1 mixture of Sabouraud broth with murine lipoprotein subfractions or lipoprotein-depleted murine plasma. After 24 and 48 hours of incubation at 37°C, aliquots of 40 μL were plated (Wasp spiral plater; DW Scientific, Shipley, UK) on Sabouraud dextrose agar. After another 24 hours of incubation at 37°C, the colonies were counted and the growth was expressed as the logarithm of colony-forming units per millilitre. Comparison between groups was done by use of the Mann-Whitney test. The Kruskal-Wallis test was used to compare the growth curves of C. albicans in depleted and nondepleted murine plasma.

Results

Growth of C. albicans in plasma of volunteers infused with rHDL. The total cholesterol and HDL concentrations in the group receiving 60 mg/kg rHDL increased from a basal level of 3.87 and 0.95 mmol/L, respectively, to 4.63 and 1.03 mmol/L at the end of the 4-hour infusion interval and to 4.72 and 1.32 mmol/L at 12 hours and 4.30 and 1.58 mmol/L at 24 hours after the start of the infusion. The total cholesterol and HDL concentrations in the group receiving 100 mg/kg rHDL increased from 3.58 and 1.12 mmol/L, respectively, to 4.38 and 1.30 mmol/L at the end of the 4-hour infusion interval and to 5.00 and 1.60 mmol/L at 12 hours and 4.50 and 2.33 mmol/L at 24 hours after the start of the infusion.

The control growth is represented by the growth of C. albicans in plasma obtained from volunteers before the start of rHDL infusion (time 0; figure 1). Infusion of 60 mg/kg rHDL had little effect on the growth of C. albicans, but C. albicans grew better in plasma obtained after infusion of 80 or 100 mg/kg rHDL (figure 1). The number of C. albicans colony-forming units grown in plasma obtained 24 hours after rHDL infusion was ~100-fold greater than that grown in control plasma. Similarly, infusion of 100 mg/kg rHDL at various infusion rates (4–12 hours) resulted in a significantly increased outgrowth of C. albicans, compared with the outgrowth of the yeast in plasma obtained before infusion (P < .05, Kruskal-Wallis test; data not shown).

Growth of C. albicans in freshly isolated human lipoprotein subclasses. As shown in figure 2A, C. albicans grew better in the lipoprotein emulsions from normal human donors than in lipoprotein-depleted plasma, suggesting that all lipoprotein subfractions are able to either enhance C. albicans growth or to inactivate fungistatic factors present in plasma. To further investigate these two possibilities, the growth of C. albicans was investigated in plasma of LDLR−/− mice.

Growth of C. albicans in plasma from hyperlipoproteinemnic LDLR−/− mice. In contrast to plasma collected from human volunteers, murine plasma inhibited the growth of C. albicans in vitro. However, this growth inhibition was significantly reduced in plasma from LDLR−/− mice [6]. After 12 hours of incubation of 10^4 cfu of C. albicans in LDLR−/− plasma, the log colony count ± SD was 3.7 ± 1.1, versus 3.2 ± 0.9 in control plasma, and after 24 hours of incubation, it was 3.4 ± 1.3, versus 3.0 ± 0.7 in control plasma (P < .05). To discern whether this effect is due to the use of lipoproteins as nutrients or to the binding and neutralization of a candidacidal factor by lipoproteins, we compared the growth curves of C. albicans in lipoprotein-depleted plasma from hyperlipoproteinemnic and normal mice. C. albicans grew significantly better in lipoprotein-depleted plasma from LDLR−/− mice than in lipoprotein-depleted plasma from the LDLR+/+ controls, although both subfractions contained similar amounts of lipoproteins (<0.01 mmol/L cholesterol) after the depletion procedure (figure 2B). This suggests that a candidacidal factor may be bound to lipo-
tein subfractions and in hyperlipoproteinemic plasma from 
LDLR\(^{-/-}\) mice. These data are in agreement with our previous 
findings in hyperlipoproteinemic LDLR\(^{-/-}\) mice, which were 
more susceptible to disseminated candidiasis because of an 
increased fungal outgrowth in their organs [6]. Although lipid 
profiles differ between mice and humans, the results of both 
studies suggest that hyperlipidemia can have deleterious effects 
by stimulating \textit{C. albicans} growth in both species.

At least two different mechanisms seem to be responsible 
for the observed effects. First, in agreement with other reports 
showing increased growth of \textit{C. albicans} in lipid emulsions 
used for parenteral feeding [7, 8], lipids themselves seem to 
promote the growth of \textit{Candida}. Cholesterol may act as a nutri-
tent for \textit{C. albicans}, as has been demonstrated for other microor-
ganisms, such as \textit{Staphylococcus aureus} [12]. After infusion 
of rHDL, natural HDL has to be formed through extraction of 
cholesterol from cell membranes and other lipoprotein subfrac-
tions, since rHDL consists of phospholipids and recombinant 
apolipoprotein A-1 without cholesterol [2]. This process re-
quires several hours, and this explains the increasing effect of 
rHDL on \textit{C. albicans} growth at later time points after infusion. 
Indeed, the total cholesterol and HDL concentrations were 
found to be higher 12 and 24 hours after the start of the infusion 
than at the end of the 4-hour infusion interval.

Second, since the growth of \textit{C. albicans} was enhanced in 
lipoprotein-depleted plasma of hyperlipoproteinemic mice, 
binding and neutralization of candidacidal factors, such as 
sphingosine [13], platelet microbicidal protein [14], or the cal-
protectin complex [15], by lipoproteins may contribute to the 
observed effect. Although no lipoproteins were present in lipo-
protein-depleted plasma isolated from either LDLR\(^{-/-}\) or nor-
mal mice, \textit{C. albicans} growth was significantly increased in 
LPDP from mice lacking LDL receptors (\(*)\) than in that from control mice (\(\square\)). Each point 
represents mean ± SD of 5 experiments. * \(P < .05\) (Kruskal-Wallis 
test).

Discussion

The results of the present study show that rHDL infusion (at 
\(\geq \! 80 \text{mg/kg}\)) increases the growth of \textit{C. albicans} in the plasma 
of human volunteers by 10- to 100-fold, compared with the basal 
growth curve in their plasma collected before rHDL administra-
tion. In contrast, there was no significant difference in the growth 
of \textit{C. albicans} in plasma before and after infusion of a moderate 
amount of rHDL (60 mg/kg). Similarly, \textit{C. albicans} growth was 
increased in the presence of freshly isolated human lipopro-

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{In vitro growth of \textit{Candida albicans} in the presence of lipoproteins. \textit{A}. \textit{C. albicans} grows better in plasma containing freshly isolated very-low-density lipoproteins (VLDL), low-density lipopro-
teins (LDL), or high-density lipoproteins (HDL), diluted 1:1 in Sa-
bouraud broth, than in lipoprotein-depleted plasma (LPDP). \textit{B}, \textit{C. albicans} grows significantly better in LPDP from mice lacking 
LDL receptors (\(*)\) than in that from control mice (\(\square\)). Each point 
represents mean ± SD of 5 experiments. * \(P < .05\) (Kruskal-Wallis 
test).}
\end{figure}
tentiates also the growth of other microorganisms, such as \textit{S. aureus} [7–9], and the clinical implication of this phenomenon for rHDL infusion warrants further investigation.

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